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THE ETHANOL LEAF EXTRACT OF *Ageratum conyzoides* AMELIORATES NIGERIAN BONNY LIGHT CRUDE OIL-INDUCED NEPHROTOXICITY IN FEMALE WISTAR RATS

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The ameliorative potentials of ethanol leaf extract of *Ageratum conyzoides* against Nigerian Bonny light crude oil-induced nephrotoxicity in female Wistar rats were investigated. Twenty female Wistar rats (120-150 g body weight) were divided into four groups of five rats each. The rats in group I served as the control group and were oral gavaged 3 ml/kg of normal saline; group II gavaged 748.33 mg/kg body weight of the extract of *A. conyzoides*, which was 20% of the LD_{50} (3741.66 mg/kg); group III gavaged 3 ml/kg body weight of Nigerian Bonny Light Crude Oil (NBLCO); this dose was calculated as 20% of the lethal dose of 14.14 ml/kg. Group IV animals were gavaged 748.33 mg/kg body weight of the extract of *A. conyzoides*, and 3 ml/kg body weight of NBLCO. In all cases, doses were applied daily for 31 days according to animal's most recent body weight.The results showed that Nigerian Bonny light crude oil significantly increase serum urea, creatinine and electrolytes except calcium where there was a significant reduction compared to the control group ($p<0.05$). But co-administration of ethanol leaf extract of *A. conyzoides* with Nigerian Bonny light crude oil caused a significant reduction in the value of the aforementioned parameters when compared with the crude oil group ($p<0.05$). It is evidenced in this study that NBLCO is a potential nephrotoxic agent as it increases serum urea, creatinine and electrolytes imbalance to significantly impaired kidney functions, which was ameliorated with co-administration of the ethanol leaf extract of *A. conyzoides*.

Keywords: *Ageratum conyzoides*, Nigerian Bonny light crude oil, nephrotoxicity, serum urea, creatinine and electrolytes.

INTRODUCTION

The kidney is a vital organ of the body with the primary function of purification (Ogbekhuemen, 2009) of plasma in complex processes involving glomerular filtration, tubular absorption and secretion. Disturbance of any kind in the aforementioned processes could result in a pathological conditions preceded by accumulation of

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wastes like urea and creatinine in the plasma because of the kidneys' inability to excrete same. Accumulation of urea and creatinine in the serum are indicative of kidney impairment, which is associated with reduction in renal efficiency as body purifier, causing accumulation of these nitrogenous products in the blood that is usually accompanied with fluid, electrolyte and acid-base disorders including hyper-kalemia. Inefficiency of the kidney can occur following injury to ultrastructure of the malphigian corpuscle, tubular epithelial cells affecting tubular transport mechanisms.

The integrity of the tubular epithelial cells is very important, as situated in these cells are ion channels and enzymatic pumps, which are made of proteins. Interference of injurious xenobiotic agents such as crude oil with the function of these channels and enzymatic pumps would ultimately affect the physiology of the kidney as a homeostatic organ (Hill, 1990). So injury of any kind to the kidney that would lead to impairment of its function would result in the accumulation of urea and creatinine that ordinarily would have been excreted. Investigations of serum urea and or creatinine levels are therefore used for diagnosis of renal disorders. Furthermore, there could also be electrolyte imbalance leading to body fluid volume distortion.

Some composition of petroleum products such as volatile nitrates, benzene and lead have been reported to produce harmful effects on lymph nodes, bone marrow and spleen (Ovuru and Ekweozor, 2004) through such interactions, there is lipid peroxidation that injured the membrane (Onwurah, 1999). They may also react with enzymes to cause inactivation through protein oxidation (Stadtman, 1990) and/or DNA strand breaks (Birnbiom and Kanabus-Kamiska, 1985; Nwanjo and Ojiako, 2007). Exposure to petroleum and its products therefore constitute a serious environmental health hazard. This has been reported to cause impairment of renal function as a result of derangement of serum electrolytes (Aryanpur, 1979; Orisakwe et al., 2004; Nwanjo and Ojiako, 2007; Uboh et al., 2009) manifesting in, blood disorders, renal damage, hepatic dysfunction and intoxication leading to serious psychotic problems, anaesthetic effects and dermatitis (Aryanpur, 1979; Nwanjo and Ojiako, 2007). The NBLCO also have been reported to cause significant impairment of kidney functions by accumulation of urea and creatinine in serum as well as electrolytes imbalance in Wistar rats (Ita and Edagha, 2016). It has been postulated that most of these xenobiotics operate through generation of radical oxidants. Imbalances between biologic pro-oxidant and antioxidant processes present toxicity crises. There are various plant-based antioxidants which are among the many elaborate, redundant nd overlapping mechanisms for combating oxidant hazards. These antioxidants include ascorbic acid, tocopherol, carotenoids and polyphenols (Halliwell and Gutteridge, 1999; Dede and Nganwuchi, 2003; Dede et al., 2003). The herbal plant *Ageratum conyzoides* is reported to be a natural source of some of these important antioxidants, which protects against harmful effects of oxidant radicals generated as a result of exposure to xenobiotic agents. This study was designed to assess the serum indices for renal function in female Wistar rat co-administered crude oil and ethanolic leaf extract of *Ageratum conyzoides* by evaluating serum urea, creatinine and some electrolytes concentration.

MATERIALS AND METHODS

Chemicals and drugs

The crude petroleum used in this study was obtained from the Exxon Mobil laboratory, Ibeno, Nigeria.

Collection of plant material

The whole plant was obtained from the Botanical Farm of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria. Specimen of the leaves was authenticated by Dr. (Mrs.) Uduak Aniema Essiett of the Department of Botany and Ecological Studies, University of Uyo, Uyo. A voucher specimen (UUH3517) was deposited at the Herbarium.

Preparation of leaf extract

The leaves of *A. conyzoides* were rinsed with distilled water and dried under shade. The dried leaves were ground into powder with an electric blender. Four hundred gram of the blended leaf sample was macerated in 700 ml 70% ethanol agitated for 10 minutes with an electric blender and left overnight in a refrigerator at 4^{o}C. The mixture was filtered with a cheese cloth and the filtrate obtained concentrated under reduced pressure using a rotary evaporator (at 37^{o}C) to about 10% of

its original volume. The concentrate was then allowed in a water bath at 37^{o}C for complete evaporation to dryness yielding 40.64 g (10.15%) of the extract.

Acute toxicity test

Acute toxicity study (LD_{50}) was estimated using Lorke's method (Lorke, 1993). A total of 25 mice weighing between 15-22 g were divided into five groups with five mice per group. Mice in the five groups were administered 3000 mg/kg, 3500 mg/kg, 4000 mg/kg, 4500 mg/kg and 5000 mg/kg of body weight respectively (intra-peritoneally). All experimental animals were observed for physical signs of toxicity such as gasping, palpitation, writhing, decreased respiratory rate, body limb and death after 24 hours. The median lethal dose of *A. conyzoides* was calculated as geometrical means of the maximum (most tolerable) dose producing 0% mortality (a) and the minimum (least tolerable) dose producing 100% mortality (b) using the formula:

$LD_{50} = \sqrt{ab}$

$LD_{50} = \sqrt{3500 \times 4000}$

$= 3741.66$ mg/kg

The acute toxicity test for the NBLCO also involved 25 mice weighing between 15-22 g were divided into five groups with five mice per group. Mice in the five groups were administered intra-peritoneally 10 ml/kg, 15 ml/kg, 20 ml/kg, 25 ml/kg and 30 ml/kg of body weight respectively.

$LD_{50} = \sqrt{10 \times 20}$

$= 14.14$ml/kg

Experimental animals

Female Albino Wistar rats weighing between 150-180g were obtained from the Animal House of the Faculty of Basic Medical Sciences University of Uyo, Uyo, Nigeria and were kept in a well-ventilated section of the Animal House. They were allowed access to feed (Chow: vital feeds, Grand Cereals Ltd, Jos) and water *adlibitum*. The animals were kept in separate experimental room and allowed to acclimatize for a period of one week before commencement of studies.

Experimental design and treatment of animals

A total of twenty (20) adult female Albino Wistar rats were randomly divided into four groups (group I, II, III and IV) of five (5) rats each. Group I served as the control and was oral gavaged 3 ml/kg body weight of normal saline. Group II was oral gavaged 748.33 mg/kg body weight of ethanolic leaf extract of *A. conyzoides*, this dose was calculated as 20% of the lethal dose (LD_{50}) of mg/kg. Group III was oral gavaged 3 ml/kg body weight of NBLCO. This dose was calculated as 20% of the lethal dose (LD_{50}) of 14.14 mg/kg, while group IV in addition to 3 ml/kg body weight of NBLCO, were supplemented with 748.33 mg/kg body weight of ethanolic leaf extract of *A. conyzoides*. In all cases, the doses were based on the rat's most recently recorded body weight. The calculated volume in milliliter (ml) was applied daily for thirty one (31) days. The experimental procedures involving the animals and their care were conducted in conformity with the approved guidelines by the Research and Ethical Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo. Nigeria.

Collection of blood sample for analysis

After thirty one (31) days of administration, the rats were anaesthetized with chloroform soaked in swap of cotton wool in a killing chamber. Blood was collected by cardiac puncture with a 5 ml sterile syringe and needle. The total volume of blood collected was 4 ml, which was transferred into plain sample bottles. This was allowed to stand for 2 hours to clot after which the serum was separated by centrifugation (RM-12 micro centrifuge, REMI, England) at 4000 rpm for 10 minutes. The serum obtained was stored at –20^{o}C until required for analysis.

Determination of serum urea

Urea kit from Dialab, (Austria) was used for the determination of urea in the serum according to method described by Veniamin and Vakirtzi (1970).

Evaluation of serum creatinine

Dialab diagnostic kit (France) was used for the determination of creatinine concentration in serum

Table 1. Comparison of some serum electrolyte concentrations in rats following exposure to NBLCO and ethanolic leaf extract of *Ageratum conyzoides*.

Groups	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{++} (mEq/L)	Cl^- (mEq/L)	HCO_3^- (mEq/L)
I	136.17 ± 2.55	3.85 ± 0.32	9.63± 0.16	100.17 ± 0.95	25.83 ± 1.01
II	151.00± 1.63[a]	5.43 ± 0.17[a]	10.63 ± 0.21	106.00± 1.81[a]	32.17 ± 1.11[a]
III	176.00 ± 6.45[a,b]	6.70 ± 0.19[a,b]	6.17± 0.61[a,b]	115.33 ± 2.03[a,b]	54.83 ± 2.63[a,b]
IV	151.00 ± 2.71[a,c]	5.28± 0.33[a,c]	8.35 ± 0.30[a,b,c]	106.33 ± 1.54[a,c]	43.33 ± 1.67[a,b,c]

a = significantly different from group I ($p<0.05$), b = significantly different from group II ($p<0.05$), c = significantly different from group II ($p<0.05$).

as described by Blass et al., (1974).

Evaluation of sodium and potassium ions in serum

TECO diagnostics kit (USA) for sodium and potassium were used to determine sodium and potassium respectively in the serum. These tests were carried out using spectrophotometry as described by Tietz (1990).

Evaluation of calcium ion in serum

Dialab diagnostics kit (Austria) for calcium was used to determine calcium in the rats' serum and urine. This test was carried out using spectrophotometry as described by Tietz (1990).

Evaluation of chloride and bicarbonate ions in serum

Agappe diagnostics kit (India) for chloride was used to determine chloride in the serum. TECO diagnostics kit (USA) for determination of carbon dioxide content in the serum. These tests were carried out using spectrophotometry as described by Tietz (1990).

STATISTICAL ANALYSIS

Data were expressed as the mean ± standard error of the mean. Statistical analysis was carried out using window SPSS package (SPSS 22.00 version). Data were analyzed using one way analysis of variance (ANOVA), results obtained were further subjected to test for least significant difference (LSD). Values of $P<0.05$ were considered significant.

RESULTS

The results of the mean values for the electrolytes obtained in this study are shown on Table 1. As would be observed NBLCO ingestion significantly increased concentration of sodium, potassium, chloride and bicarbonate in serum with respect to groups I and II (the control and *A. conyzoides* groups respectively) ($p<0.05$). Co-administration of *A. conyzoides* to group IV animals significantly reversed the values of these aforementioned electrolytes with respect to NBLCO-treated group (group III) ($p<0.05$). Similarly, NBLCO significantly reduced calcium concentration compared with groups I and II (the control and *A. conyzoides* groups respectively) ($p<0.05$), co-administration of *A. conyzoides* significantly raised serum concentration of calcium higher than NBLCO-treated group (III) ($p<0.05$), but significantly lowered than groups I and II (the control and *A. conyzoides* groups respectively) ($p<0.05$).

Serum creatinine levels in control and various experimental groups.

The results of the mean values for the urea and creatinine obtained in this study are shown on Table 2. The mean urea level in NBLCO treated rats was significantly higher than groups I and II (the control and *A. conyzoides* groups respectively) ($p<0.05$). Co-administration of *A. conyzoides* significantly increased serum urea level higher than groups I and II ($p<0.00\%$) though, the co-administration significantly reduced urea level compared with NBLCO treated group ($p<0.05$).

Table 2. Comparison of serum urea and creatinine in rats following exposure to NBLCO and ethanolic leaf extract of *Ageratum conyzoides*.

Groups	Urea (µmmol/L)	Creatinine (µmmol/L)	Urea/Creatinine
I	13.15 ± 0.23	0.32± 0.03	0.787± 0.016
II	20.40 ± 1.18[a]	0.55± 0.04[a]	0.738± 0.008[a]
III	29.48 ± 1.48[a,b]	1.00 ± 0.09[a,b]	0.794± 0.007[b]
IV	26.35 ± 0.75[a,b,c]	0.75± 0.04[a,b,c]	0.774± 0.005[b]

a = significantly different from group I ($p<0.05$), b = significantly different from group II ($p<0.05$), c = significantly different from group III ($p<0.05$)

The results on mean creatinine level followed the same trend as in urea where NBLCO treated rats was significantly higher than groups I and II (the control and *A. conyzoides* groups respectively) ($p<0.05$). Co-administration of *A. conyzoides* significantly increased serum creatinine level higher than groups I and II ($p<0.00\%$) though, but significantly lowered creatinine level compared withNBLCO treated group ($p<0.05$).

DISCUSSION

Nephrotoxicity and ameliorative effects of NBLCO and *Ageratum conyzoides* respectively have been demonstrated in this study. The accumulation of wastes like urea and creatinine in serum; and distortion in electrolyte distribution resulting in accumulation of sodium, potassium, chloride and bicarbonate in serum in this study is suggestive of nephrotoxicity potentials of NBLCO. The significant elevation of serum sodium, potassium, chloride and bicarbonate concentration, indicates possible distortions of the membrane integrity and functions by the toxicants in NBLCO. Ingestion of crude oil has been reported to induce oxidative damage to trans-membrane ATPase activity, thereby inducing cell lysis (Brovelli et al., 1977; Ita et al., 2013). Furthermore, elevated lipid peroxidative activity interfered with the membrane architecture to increase membrane permeability leading to cell lysis. A similar report on erythrocyte membrane has been reported by Ita et al (2013), that ingestion of NBLCO cause erythrocyte haemolysis in rats. Brovelli et al, (1977) had reported that free radicals have the propensity to induce oxidative damage to membrane ATPase and in turn increase potassium efflux from the cell; this corroborates the findings of this study.

Kidneys are the purifier of plasma that flow to them so injury to them is usually indicated with accumulation of nitrogenous waste like urea and creatinine in serum which indicate kidney impairment, this may be accompanied by fluid and electrolyte imbalance as evidently observed in this present study. It is interesting to note that the body's dependency on the kidneys to excrete urea makes it a useful analyte to evaluate renal function. Accumulation of urea and creatinine in serum as reported in this study has been postulated to suggest renal impairment which might be mediated through oxidative stress (Traynor et al., 2006). Accumulation of serum urea also indicates high protein catabolic rate in the body that could be coupled to inadequate excretion by the kidneys.

Significant renal damage as in renal disease or injury is associated with reduced glomerular filtration rate, which might possibly compromise creatinine clearance leading to accumulation of creatinine in plasma as observed in this study. When integrity of the malphigian corpuscle is comprised by either disease condition or xenobiotic insults, in addition to other factors glomerular filtration rate is evidently expected to reduce. Recent report on the histological section of the kidney following ingestion of the NBLCO has been reported to cause serious distortion in the cyto-architecture of the malphigian corpuscle of the kidneys (Ita and Edagha, 2016) leading to inadequate filtering of urea and creatinine in the tubular content.

The co-administration of *A. conyzoides* was observed to have ameliorated the hazardous effects of NBLCO reversing the aforementioned parameters. The ethanol leaf extract of *A. conyzoides* may have been able to cause the reversal of effects because of its many bioactive

chemical compounds, some of which are powerful antioxidants. These chemical compounds constitute the secondary metabolites, which are responsible for most pharmacological activities of the plant (Agbafor et al., 2010). Importantly, the antioxidant property of the plant is reported to be associated with the secondary metabolite, saponins (Olaleye, 2007).

It is evidenced in this study that NBLCO is a potential nephrotoxic agent as it increases serum urea, creatinine and electrolytes imbalance to significantly impaired kidney functions, which was ameliorated with co-administration of the ethanol leaf extract of *Ageratum conyzoides*.

CONCLUSION

It is therefore concluded that co-administration of Ageratum conyzoides ameliorates NBLCO induced nephrotoxicity in rats.

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